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Original Research

Quercetin regulates hepatic cholesterol metabolism by promoting cholesterol-to-bile acid conversion and cholesterol efflux in rats

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ABSTRACT

Quercetin, a common member of the flavonoid family, is widely present in plant kingdom. Despite that quercetin is implicated in regulating cholesterol metabolism, the molecular mechanism is poorly understood. We hypothesized that quercetin regulates cholesterol homeostasis through regulating the key enzymes involved in hepatic cholesterol metabolism. To test this hypothesis, we compared the profile of key enzymes and transcription factors involved in the hepatic cholesterol metabolism in rats with or without quercetin supplementation. Twenty male Wistar rats were randomly divided into control and quercetin-supplemented groups. Serum total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total bile acids in feces and bile were measured. Hepatic enzymatic activities were determined by activity assay kit and high-performance liquid chromatography–based analyses. The messenger RNA (mRNA) and protein expressions were determined by reverse transcriptase polymerase chain reaction and Western blot analyses, respectively. The results showed that the activity of hepatic cholesterol 7α-hydroxylase, a critical enzyme in the conversion of cholesterol to bile acids, was significantly elevated by quercetin. The expression of cholesterol 7α-hydroxylase, as well as liver X receptor α, an important transcription factor, was also increased at both mRNA and protein levels by quercetin. However, quercetin exposure had no impact on the activity of hepatic HMG-CoA reductase, a rate-limiting enzyme in the biosynthesis of cholesterol. We also found that quercetin treatment significantly increased ATP binding cassette transporter G1 mRNA and protein expression in the liver, suggesting that quercetin may increase hepatic cholesterol efflux. Collectively, the results presented here indicate that quercetin regulates hepatic cholesterol metabolism mainly through the pathways that promote cholesterol-to-bile acid conversion and cholesterol efflux.

Abbreviations: ABCA1, ATP binding cassette transporter A1; ABCG1, ATP binding cassette transporter G1; CYP7A1, cholesterol 7α-hydroxylase; FXR, farnesoid X receptor; HCO, 7α-hydroxy-4-cholesten-3-one; HDL-C, high-density lipoprotein cholesterol; HMG CR, HMG-CoA reductase; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LXRα, liver X receptor α; SREBP, sterol regulatory element-binding protein; TC, total cholesterol; TG, triglyceride.

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1. Introduction

Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a common member of the flavonoid family, is present widely in vegetables, fruits, tea, and red wine [1]. Quercetin acts as a scavenger of superoxide and hydroxyl radicals, a metal chelator, or an inhibitor of lipid peroxidation [2–5]. Recently, several studies have demonstrated that quercetin is effective in regulating cholesterol metabolism [6–8]. Our previous study showed that exposure of rats to a quercetin-supplemented AIN-93 diet changed serum lipid profiles by increasing serum low-density lipoprotein cholesterol (LDL-C) [9]. Another study conducted by Tang et al [10] found that quercetin treatment significantly reduced the cholesterol contents of the liver, heart, kidney, and small intestine in rats. The molecular mechanisms underlying these biological actions of quercetin are currently unclear.

Cholesterol is ubiquitously present in all animal cells and plays essential roles in many cellular events. As one of the major components of cell membrane, cholesterol strengthens cell membrane integrity and influences lipid fluidity [11]. In addition to being an important structural component of membrane, cholesterol also plays an important role in cell signaling and intracellular transport [12,13]. Moreover, cholesterol can be converted to other essential molecules, such as bile acids, vitamin D, and sterol hormones [14]. Despite its well-established physiological functions, high level of blood cholesterol is strongly associated with the development of atherosclerosis [15]. Low-density lipoprotein cholesterol, often termed as *bad cholesterol*, may lead to coronary artery disease due to its contribution to the formation of atheroma [16].

The liver is the major organ for the biosynthesis of cholesterol, as well as the conversion of cholesterol to bile acids [17]. Cholesterol biosynthesis is controlled by a cascade of pathways, in which HMG-CoA reductase (HMG CR) plays a rate-limiting role [18]. Transcription of HMG CR is controlled by sterol regulatory element-binding protein (SREBP) [19,20], which translocates from Golgi apparatus after being truncated by site 1 and 2 protease [18]. Low-density lipoprotein receptor (LDLR), another protein involved in cholesterol metabolism, functions mainly in scavenging circulating LDL from the bloodstream and is also regulated by the SREBP pathway [18–20]. Cholesterol 7α-hydroxylase (CYP7A1) plays a critical role in the conversion of cholesterol to bile acids, which is regulated by liver X receptor (LXR) and farnesoid X receptor (FXR) [21]. Liver X receptor also participates in regulating cholesterol homeostasis by inducing the expression of ATP binding cassette transporter A1 (ABCA1) and ATP binding cassette transporter G1 (ABCG1), which are involved in the efflux of cellular cholesterol [22].

Based on the literature and our previous studies [9,10], we hypothesize that quercetin is involved in modulating the pathways of hepatic cholesterol metabolism. Hence, we investigated the effects of quercetin on a number of critical enzymes and transcription factors in hepatic cholesterol metabolism. The messenger RNA (mRNA) and protein expression levels and activities of these enzymes and related factors, including CYP7A1, HMG CR, LXRα, FXR, SREBP-2, ABCA1, ABCG1, and LDLR, were measured. Our data suggest that quercetin promotes cholesterol-to-bile acid conversion and cholesterol efflux, whereas it has a little impact on the biosynthesis of cholesterol.

2. Methods and materials

2.1. Animals and diets

Twenty male Wistar rats weighing 180 to 200 g were obtained from the Experimental Animal Center of the Chinese Academy of Military Medical Sciences (Beijing, China). The rats were housed individually in stainless steel cages at 23°C ± 1°C and 40% to 60% relative humidity. The light/dark cycle was alternated every 12 hours. The experimental protocol was approved by the ethics committee of Tianjin Institute of Health and Environmental Medicine. All procedures were performed in accordance with the current Chinese legislations on the care and use of laboratory animals. To acclimate to the facility, the rats were initially fed the AIN-93G diet for 1 week. The AIN-93G diet was prepared by the guideline described elsewhere [23]. The ingredient composition of the AIN-93G diet is listed in Table 1. The rats were randomly divided into control and quercetin-supplemented groups. Quercetin was purchased from Sigma (St Louis, MO, USA). Because quercetin is relatively unstable when exposed to atmospheric oxygen, it was stored at –20°C and was added to the diet directly before the rats was fed to minimize possible degradation.

Rats in the control group were fed the AIN-93G diet and those in the quercetin-supplemented group were fed the AIN-93G diet containing 0.4% quercetin for 5 weeks. Dietary supplementation of quercetin was chosen at 4.0 g/kg to induce a significant change in serum LDL-C level based on the literature and our previous studies [9,10,24].

All animals had free access to their diets and tap water. Food intake was recorded daily and body weight was measured weekly. At the end of the experiments, all rats were fasted overnight. Blood samples were obtained from the orbital plexus under ether anesthesia and serum was collected after centrifugation. All rats were euthanized by ether inhalation.

2.2. Measurement of serum lipids

The levels of serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and LDL-C were measured using a HITACHI 7100 automatic biochemical analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). The reagent kits were purchased from Biosino Biotechnology and Science Inc, Beijing, China. The procedures described by the instructions attached were strictly followed.

Table 1 – The ingredient composition of the diet fed to rats

Ingredient	g/kg diet
Cornstarch	465.692
Casein (>85% protein)	140.0
Dextrinized cornstarch (90%-94% tetrasaccharides)	155.0
Sucrose	100.0
Soybean oil	40.0
Fiber	50.0
Mineral mix (AIN-93M-MX)	35.0
Vitamin mix (AIN-93-VX)	10.0
L-Cystine	1.80
Chloride choline (41.1% choline)	2.50
Tert-butylhydroquinone	0.008